STUDIES IN PLANT METABOLISM. VI. EFFECT OF 2,4-D ON THE METABOLISM OF ASPARTIC ACID AND GLUTAMIC ACID IN THE BEAN PLANT[1,2,3]

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In studying the effect of 2,4-D treatment on the chemical composition of bean plants, Sell et al (7) reported that the stem of 2,4-D treated plants contained almost twice as much crude protein as do stems of control plants. Later, Weller et al (9) found that 2,4-D treatment caused a slight decrease in crude leaf protein while the crude root protein remained approximately the same. The amino acid composition in the crude protein from 2,4-D treated stems were different from those of the control plants. A small difference was noted in the leaf and root proteins. These data suggested to the authors that a change had occurred in the character of the protein. Using potato tubers, Payne et al (5) found that 2,4-D caused an increase in free glutamic acid and a decrease in eleven amino acids including aspartic acid. The decrease was explained as an increased catabolism of the free amino acids. In a preliminary investigation on the effects of 2,4-D 2,4,5-T and indoleacetic acid treatment, we have found that the amounts of total aspartic and glutamic acids decreased in both the stems and roots of 2,4-D and 2,4,5-T treated plants, with the greatest change occurring in the stems. Two possible explanations may be given: 1) the 2,4-D treatment might inhibit the synthesis of these amino acids or 2) this treatment might increase the rate of oxidation of the amino acids.

In order to clarify this point, the present investigation, using carbon 14 as a tracer to study the rates of synthesis of both glutamic acid and aspartic acid in 2,4-D treated and control plants was carried out.

MATERIALS AND METHODS

TREATMENT OF PLANTS: Bean plants (*Phaseolus vulgaris* var. Black Valentine) were grown in potted soil, four plants to a pot, under greenhouse conditions. The plants were allowed to grow until the primary leaves were fully unfolded and the second internode well defined. At this time three uniform pots were chosen and two plants in each pot were treated on the midrib of one primary leaf with 50 μgm of 2,4-D dissolved in an ethanol solution containing 0.1 % of the free 2,4-D acid and 0.5 % Tween-20. The other two plants in each pot were left untreated as control plants.

A closed system was prepared as shown in figure 1. This system consisted of a bell jar large enough to contain one set of four plants, a column of calcium chloride to absorb the excess moisture, a generator to produce radioactive CO_2 from $BaC^{14}O_3$ after the system was sealed, a Geiger-Müller tube to detect the radioactivity, and a pump to circulate the atmosphere.

One day after treatment, the first set of bean plants was placed in the closed system and exposed to an atmosphere containing $C^{14}O_2$ for a period of 12 hours in light and 12 hours in darkness. Two 40-watt fluorescent lamps which have a light intensity of 200 fc were set 6 inches from the bell jar on opposite sides. The radioactivity readings of the atmosphere were taken periodically in order to check the amount of radioactivity absorbed by the plants. The second and third sets of bean plants were exposed to $C^{14}O_2$ in a similar manner, three days and seven days, respectively, after the treatment with 2,4-D. The amounts of $BaC^{14}O_3$ used for these three experiments were 4.3 mg, 6.3 mg, and 5.1 mg respectively (20.6 μc/mg).

After exposure to radioactive CO_2, the entire plants were harvested and dried overnight at 55° C in a vacuum oven. The samples were then weighed, ground and stored in an evacuated desiccator.

RADIOACTIVITY MEASUREMENTS: Weighed aliquots of each sample were oxidized by dry combustion (2). The carbon dioxide was quantitatively absorbed in carbonate-free sodium hydroxide solution and precipitated as barium carbonate. The radioactivity of the barium carbonate samples was measured with a thin mica window Geiger-Müller counter (1.9 mg/cm^2) attached to a scaler in the usual manner. All measurements were corrected to zero thickness.

After the paper chromatograms were developed (see Isolation and Analyses) and dried, the radioactivity of the amino acids was determined. The paper strips were counted at centimeter intervals through a one centimeter slit. The Geiger counter was placed as close as possible to the strip to increase the counting efficiency. Several one centimeter sections of the paper chromatograms containing various amounts of radioactivity were oxidized, and the radioactivity was redetermined as barium carbonate. These comparison values were plotted to determine a correction curve. Thus the radioactivity found in the paper chromatograms could be expressed as $BaCO_3$ activity.

ISOLATION AND ANALYSES: Weighed amounts of each sample (100 mg) were autoclaved with 10 ml of 2 *N* HCl in sealed tubes for 10 hrs at 15 lbs pres-

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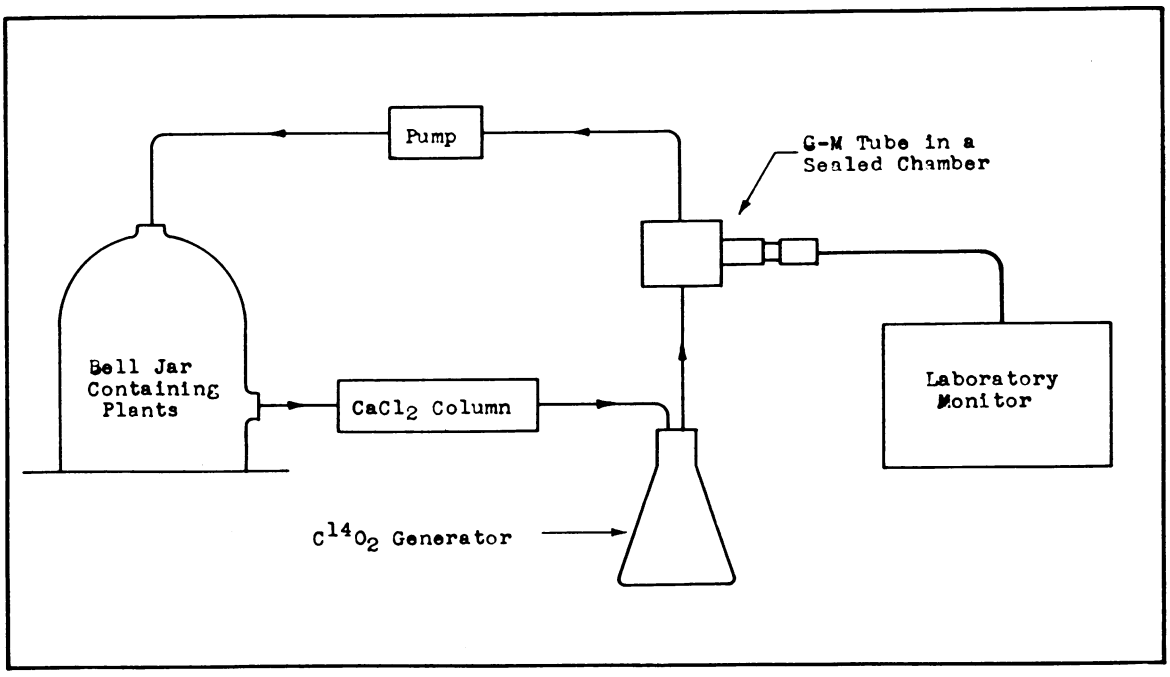

FIG. 1. Block diagram of the sealed system used for $C^{14}O_2$ absorption.

sure. The excess hydrochloric acid was removed from the hydrolysate by repeated addition of water and evaporation to dryness in vacuo. The hydrolysate was made up to 100 ml and the residue was removed by filtering through a dry filter.

The acidic compounds were separated from the neutral and basic compounds by means of ion-exchange columns of Amberlite IR-4B as follows: Several glass columns, 16 mm in diameter, were filled to a height of 25 cm with resin in the basic form. The columns were back washed, charged with 4 % NH_4-OH, and washed with distilled water until neutral.

The corresponding pairs of treated and control samples were run simultaneously to reduce the errors due to differences in operation. An aliquot of each solution was allowed to pass through a resin column at the rate of approximately 0.5 ml per minute. When the last of the solution reached the top of the resin bed, the column was washed with distilled water, allowing no break in the continuity of the liquid phase. This washing was continued until no activity was detected in the effluent. An excess amount of 4 % HCl was passed through the column to elute the acidic compounds (including aspartic and glutamic acids) from the resin. The HCl was again removed from the effluent by the method described previously. The samples were then diluted to 25 ml.

The aspartic and glutamic acid were separated by means of paper chromatography. Aliquots of the solutions were applied quantitatively as spots or lines to strips of Whatman #1 filter paper (1″ × 22″). These strips were developed in aqueous phenol for 18 hours by the descending front method and were dried at room temperature after developing.

After the radioactivity of the unknown strips had been measured, quantitative determination of the amino acids present was made by Block's method (1) using ninhydrin as a color reagent. The relative color density was measured with a Photovolt Electronic Densitometer, model 525, using a blue filter. The results were compared with a standard curve prepared in the similar manner from known amounts of amino acids. If carefully conducted, accuracies of ± 5 % are readily obtained by this method.

RESULTS AND DISCUSSION

Approximately 90 % of the radioactivity was absorbed by the bean plants in the first 8 hours of exposure. The amount of C^{14} incorporated into each plant sample is given in table I. It is interesting to note that the 2,4-D treated plants absorbed much less radioactive CO_2 than did the control plants. Furthermore, this difference in absorption which is shown in table I as the ratio of total radioactivity, is relatively the same for all three experiments. (T/C = 0.615, 0.622 and 0.615 for 1st, 3rd and 7th day samples respectively.) These results, which indicate that 2,4-D treatment reduces the rate of photosynthesis, agree closely with the work reported by Freeland (3).

The results of the chemical analyses are given in table II. The total aspartic acid and glutamic acid content of each plant sample is shown on the left side of the table. Since the number of plants in each experiment was small and the weights of the plant varied somewhat, the percentage of amino acids in each sample was calculated to obtain comparable values. These data are shown on the right side of table II.

In the first-day samples, the percentages of both

TABLE I

EFFECT OF 2,4-D TREATMENT ON PHOTOSYNTHESIS IN BEAN PLANTS AS DETERMINED BY ANALYSES OF C^{14} IN PLANT TISSUE

SAMPLE	WT OF PLANT SAMPLE, MG	TOTAL RADIO-ACTIVITY, CPM × 10^3 **	$BaC^{14}O_3$ USED, MG *	SPECIFIC ACTIVITY, CPM × 10^3/MG
		1st Day		
Treated	864.6	3,817		4.41
Control	688.1	6,203	4.3	9.01
T/C		0.615		
		3rd Day		
Treated	893.4	6,419		7.18
Control	879.4	10,312	6.3	11.73
T/C		0.622		
		7th Day		
Treated	876.3	4,929		5.62
Control	1079.9	8,010	5.1	7.42
T/C		0.615		

* Specific activity of $BaC^{14}O_3$ is 20.6 μc/mg.
** The activity was measured by a thin mica window G-M counter (1.9 mg/cm²).

amino acids in whole plants were essentially the same in both control and treated plants, while in the 3rd and 7th day samples a great decrease in these amino acids was noted in the 2,4-D treated plants.

Sell et al (7) and Weller et al (9) have reported the effect of 2,4-D treatment on changes in chemical composition of the leaves, stems and roots of the red kidney bean plants. From their more complete results on the analyses of eleven amino acids, they have suggested that 2,4-D treatment causes a change in the character of the plant protein. These findings of the change in aspartic acid and glutamic acid concentration agree closely with their results.

The results of the radioactive analyses are summarized in table III. On the left side of the table, the total radioactivity of aspartic and glutamic acids in each plant sample is shown. Since different amounts of radioactive barium carbonate were used in the three experiments and since the rate of fixation of the radioactivity was greatly reduced in the treated plants, the percentage incorporation of C^{14} into each amino acid was calculated. These percentages, as shown on the right side of the table, indicate that the amounts of C^{14} incorporated into aspartic and glutamic acids were approximately 3 to 4 times greater in the treated than in the control plants, with the most pronounced effect on the third day. It is also interesting to note that the incorporation of C^{14} into glutamic acid increased with the age of bean plants while a decrease was found in the aspartic acid.

If the metabolic cycling of C^{14} was unaffected in the treated plants, the amount of radioactivity incorporated into the amino acids should indicate the difference in the rate of synthesis during the experimental period. Since 2,4-D treatment increased the incorporation of C^{14} into aspartic and glutamic acids, it is logical to conclude that the rate of synthesis of these two amino acids had been increased. However, the total quantity of these two amino acids was markedly decreased in the treated plants particularly in the third and the seventh day samples. Therefore, 2,4-D treatment must increase the rate of oxidation or catabolism of these amino acids, and this increase must exceed the increase in the rate of synthesis. Rebstock et al (6) and Freiburg (4) found that 2,4-D caused increases in proteinase and polypeptidase activities which lends some support to these findings.

Carbohydrate and sugar reserves in bean plants are readily depleted following 2,4-D treatment, as reported by several workers (7, 8, 9) and the synthesis of aspartic and glutamic acids is increased as indi-

TABLE II

EFFECT OF 2,4-D TREATMENT ON THE AMOUNTS OF ASPARTIC AND GLUTAMIC ACIDS IN BEAN PLANTS

TOTAL AMOUNT IN PLANT TISSUE (μGM)				% AMINO ACID IN PLANT TISSUE			
ASPARTIC ACID		GLUTAMIC ACID		ASPARTIC ACID		GLUTAMIC ACID	
TREATED	CONTROL	TREATED	CONTROL	TREATED	CONTROL	TREATED	CONTROL
			1st Day				
3644	3016	2114	1569	0.421	0.438	0.245	0.228
			3rd Day				
3858	4431	1336	2351	0.432	0.504	0.150	0.267
			7th Day				
2178	4117	1234	2952	0.244	0.381	0.141	0.273

All values are averages of triplicate determination.

TABLE III

EFFECT OF 2,4-D TREATMENT ON THE INCORPORATION OF C^{14} INTO ASPARTIC AND GLUTAMIC ACIDS IN BEAN PLANTS

TOTAL RADIOACTIVITY, CPM × 10^3				% INCORPORATION OF C^{14}					
ASPARTIC ACID		GLUTAMIC ACID		ASPARTIC ACID			GLUTAMIC ACID		
TREATED	CONTROL	TREATED	CONTROL	TREATED	CONTROL	T/C	TREATED	CONTROL	T/C
				1st Day					
26.9	16.0	32.5	16.7	0.71	0.26	2.73	0.85	0.27	3.15
				3rd Day					
57.3	22.1	113.8	51.0	0.89	0.21	4.15	1.77	0.50	3.58
				7th Day					
13.4	6.4	91.3	57.3	0.27	0.08	3.40	1.85	0.72	2.59

All values are averages of triplicate determination.

cated by this work. It would seem, therefore, that during photosynthesis the ordinary reaction from C_3 to C_6 sugar is greatly inhibited, more carbon dioxide enters the Kreb's cycle through the condensation of C_3 to C_4 units, which subsequently increases the synthesis of amino acids. This increase in the synthesis of aspartic and glutamic acids might probably result from the normal process of transamination, and the decrease in total amounts of these amino acids during this seven day period could then be solely due to an increased catabolism of the protein. The carbon fragments from this oxidation might not return for resynthesis of amino acids as suggested by Payne et al (5) in her experiment with potato tubers. In view of the fact that the amount of aspartic and glutamic acid, measured by this method, included both free and bonded amino acids as well as from the hydrolysis of asparagine and glutamine, it is impossible at present to determine whether the primary effect of 2,4-D treatment is on the free amino acids or on the amides. However, this result undoubtedly indicated that 2,4-D treatment may affect both the synthesis and the oxidation of these two amino acids in bean plants.

Summary

1. The rate of photosynthesis in bean plants was markedly reduced by 2,4-D treatment. The rate of reduction remained essentially constant over the seven day period after treatment.

2. The incorporation of C^{14} into aspartic and glutamic acids was 3 to 4 times greater in the treated plants than in the control plants. The most pronounced effect was observed in plants harvested on the third day.

3. Total amounts of aspartic acid and glutamic acid decreased in the treated plants, especially in the plants harvested on the seventh day after treatment. A possible explanation for the increased incorporation of C^{14} and more rapid loss of aspartic and glutamic acids is discussed.

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PARTICULATE ADENYLIC KINASE IN HIGHER PLANTS[1,2]

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Adenylic kinase (AK) which catalyzes the reversible reaction

$$(1) \qquad 2ADP \rightleftharpoons ATP + AMP$$

was first described by Colowick and Kalckar (1) and named by them myokinase. The name was assigned because rabbit and frog skeletal muscle were the richest sources of the enzyme. Kotelnikova (2) has since shown that the enzyme is widely distributed in the animal organism, being present in liver, kidney, and erythrocytes in addition to muscle. The enzyme has been shown to be associated with the mitochondria in rat and mouse liver preparations (3, 4, 5).

As far as the plant kingdom is concerned, the enzyme has been described in yeast (6) but no reference is to be found in the literature concerning higher plants. However, Loomis (7), in his doctoral dissertation, and Stumpf (31) in a review present evidence for the presence of the enzyme in extracts of acetone powders of pumpkin seedlings and manroot leaves and also mention a personal communication from Axelrod, Bandurski and Campbell stating they had shown adenylic kinase (AK) activity in plant tissue. The present study demonstrates the presence of an adenylic kinase associated with the mitochondria of cotyledons from peanut and lupine seedlings and the chloroplasts from spinach and tobacco leaves. Some

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